minutes. A final elongation cycle at 68°C for 7 minutes was also included. An MJ Research PTC thermal cycler was used for the PCR reaction. After the PCR reaction was carried out, the plasmid template in each of the PCR reactions was digested with the *DpnI* enzyme, which cleaves the methylated DNA template and not the PCR product.

For each library, 1μL of the *DpnI* digested PCR reaction was transformed by electroporation into TOP10 one-shot electrocompetent cells from Invitrogen. The electroporation was conducted using a BIORAD electroporator. A fifth of the transformation mix was plated on LB plates containing 50μg/mL kanamycin (kan) and the remaining mix was plated on LB plates containing 50μg/mL kan and 0.5μg/mL moxalactam (mox; obtained from Sigma). Between 2000 and 4000 transformants were obtained per transformation based on the number of colonies observed on the kan plates. Several transformations were carried out to obtain 21000 and 54000 colonies for the IRL1 and IRL2 libraries respectively. Those transformants that grew on plates containing mox were streaked for single colonies on LB plates containing 50μg/mL kan and 0.5μg/mL mox. A single colony from each of the mox-resistant clones was used to inoculate 200μL of LB containing kan in a 96 well microtiter plate. The plate was grown at 37°C with shaking for 18 hours, and each of the cultures in the wells was diluted 10,000-fold into 12 microtiter plates containing LB with different concentrations of mox (0 to 100μg/mL). Kanamycin was also added to the media to maintain selection for the *ampC* pAL20 plasmid. After incubation at 37°C with shaking for up to 21 hours, the absorbance of the cells grown in each well was measured at 600nm. The fold increase in mox resistance was calculated based on the extent of growth of cells containing the wild type *ampC* gene. Plasmids were extracted for sequencing from all library clones that had a mox resistance of greater than 2.5 fold compared to wild type.

Example 4. Generation of a Conservation Index as a Constraint Vector

A conservation index may be defined as a measure of the degree of conservation at each position in a multiple sequence alignment. A conservation index algorithm developed by Novere et al. (Biophys. Journal v.76 , p. 2329-2345, May 1999) was used to generate a conservation index based on the alignment of the ampC proteins. A conservation index was assigned at each position in the alignment according to the equation:

$$CI = \frac{\sum_{i=1}^{N} \sum_{j=i+1}^{N} S_{ij}}{\sum_{i=1}^{N} \sum_{j=i+1}^{N} S_{ij}}$$

where N is the number of sequences in the alignment, S_{ij} are the global similarities of the ith and jth sequences, and s_{ij} is the relevant similarity matrix element for the sequences i and j at the given position. The default similarity matrix from the Wisconsin package program GAP (Devereux et al., 1984) can be used, rescaled to [0-100]. The resulting values range from 0 to 100. A score of 100 indicates absolute conservation.

Although the invention has been described in some detail with reference to the preferred embodiments, those of skill in the art will realize, in light of the teachings herein, that certain changes and modifications can be made without departing from the spirit and scope of the invention. Accordingly, the invention is limited only by the claims.